



(11) **EP 0 791 653 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
27.08.1997 Bulletin 1997/35

(51) Int. Cl.⁶: **C12N 15/12**, **C07K 14/735**,
A61K 38/17

(21) Application number: 97106398.7

(22) Date of filing: 24.05.1989

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(30) Priority: 27.05.1988 US 199513
19.01.1989 US 300039

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
89305241.5 / 0 343 950

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Remarks:

This application was filed on 16 - 04 - 1997 as a divisional application to the application mentioned under INID code 62.

(54) **Human Fc-gamma receptor III**

(57) Soluble and membrane-bound human Fc γ RIII and variants are provided, together with nucleic acids encoding the same. Also described are pharmaceutical compositions containing Fc γ RIII or variants and the use of these proteins for treatment of immune thrombocytopenic purpura.

[illegible]

70 +1 90

CTACCTTTTCAGCTGGCCATCGGGACTGGAAGATGTTCCCAAAAGGCTGTGGT
LeuValSerAlaGlyMetGluActGluAspLeuProPheAspLeuValVal
170 180

AAGTGGCAGGGGAGCCTACTCCOCTTGAGGACAATTTCGACACAGTGTGTT
LysCysGlnGlyAlaTyrSerProGluAspAsnSerThrGlnTrpPhe
270 280

ACAGCTCGAAGCAGAGCTCGAGAGTACAGCTGCCACAGCAAACTCCTCCA
ThrValAspAspSerGlyGluTyrArgCysGlnThrAsnLeuSerT
370 290

CGCCCTCGGTGGTGTTTCAAGCAGGAAGCCOCTATTGACCTGAGGTG
AlaProArgTrpValPheLysGluGluAspProIleHisLeuArgCys
470 480

AAAGACAGGAAGTATTTTCATCATATAATTCTGCATTCACATATCCAAAA
LysAspArgLysTyrPheHisIleAsnSerAspPheHisIleProLys
570 580

AATGTGTGTTGAGAGACTGTGAACATCCACATCAGTCAAGGCTTTGG
AsnValSerSerGluThrValIleAsnIleThrIleThrGlnGlyLeuA
670 680

TTGTGTATGCTACTGCTTTTGGAGTGGACACAGGACTATATTTCTG
LeuValMetValLeuLeuPheAlaValAspThrGlyLeuTyrPheSer
770 780

AATGGAGAAAAGCAOCCCTCAAGACAAATGACCCOCCATCCCATGGGAGT

ATCATGTCGAGGCGCTGTTCTACAAGGACGAGGAAACATAGAATTCTCAGA
AAGCGCAGCTTTCTCAAGACGAGGAAACCCOCCAGTGACTGATCGATTC
1000
000CCTCAGCTAAAAACAACAATACAGCGGTACAGCTAGTGAATGCTTTAAA
ATAATATTATCTGTAAACAAATGAGTAAGTAAATTAAGATTGAGGG
1200
00ATGAAACAGTGGTGGAGAAATAGCAGCGTATCGTCAAGATTTGATCTAA
OCCGTATGTGGAATTAAGCAAGAAATGTCAGCGTACAGTGAAGTAAATG
1400
00CTCTAATGCTAGGATGATAAGAAATGTGCTCTATGACAGGATGAGTGAAGA
TCCAACATTAAGGTAAGTCAAGCAAGAAATATGTCAGCAAGTAAGATGAG
1600
00GAGGAGAGTGAAAATGAGTTTGTGGCCAGAGGTTAAGTAATTAAGAGG
TGAGCTTGTGCTATGCTATTTATTAACAACAAATGTTTAAATAACCAAT
1800
00TACGTACGATTCGAATGAGGTGGGAGTGGAGAGCAAGCAATTTGTTGCTT
CTTGCTTGCATAAAGCACTGGAAGTGAAGTGAAGTGAAGTGAAGTGAAGT

FIGURE 1 PART B

FIGURE 1 PART A

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Application No.: 10/756,153
Attorney Docket No.: 13783-105015
References
B4

Description

Field of the Invention

The invention relates generally to therapeutic compounds, and more particularly to soluble and membrane-bound forms of a low-affinity receptor for human immunoglobulin G, nucleic acids encoding the same, and diagnostic and therapeutic uses of such receptors.

BACKGROUND

Receptors for the Fc portion of immunoglobulin G (IgG) play a central role in cellular immune defenses. Three types of such receptors have been identified: A 72 kilodalton (kD) receptor with high affinity for monomeric IgG is found on monocytes and some macrophages, a 40 kD receptor with low affinity for monomeric IgG is found on monocytes, neutrophils, eosinophils, platelets and certain human tumor-cell lines, and a 50-70 kD receptor with low affinity for monomeric IgG is found on neutrophils, eosinophils, natural killer cells, and macrophages. These three types of Fc γ receptor are referred to as Fc γ RI, Fc γ RII, and Fc γ RIII, respectively: Unkeless et al., *Ann. Rev. Immunol.*, Vol. 6, pgs. 251-281 (1988).

It is believed that Fc γ RIII-mediated removal IgG-coated platelets plays an important part in the pathogenesis of immune thrombocytopenic purpura (ITP), a platelet-deficiency condition characterized by excessive bleeding: von dem Borne, pgs. 222-256, in *Immunohaematology*, Engelfriet et al., eds. (Elsevier, Amsterdam, 1984). Clarkson et al., *New England J. Med.*, Vol. 314, pgs. 1236-1239 (1986), report that the infusion of ligand-blocking anti-Fc γ RIII antibody into a patient with refractory ITP resulted in a transient increase in platelet count. This observation suggests that the most deleterious manifestation of ITP could be temporarily ameliorated by the administration of agents that block or compete with Fc γ RIII for binding sites on IgG-coated platelets.

In a separate area of clinical immunology, elevated serum levels of aggregates consisting of immunoglobulin and antigen (so-called "immune complexes") have been correlated with a wide variety of disorders, particularly autoimmune diseases, such as systemic lupus erythematosus (SLE), and rheumatoid arthritis. The level of such complexes has become an important diagnostic for presence of autoimmune disorders; e.g. Theofilopoulos et al., *Am. J. Pathol.*, Col. 100, pgs. 531-591 (1980).

Present assays for the serum level of immune complexes include solid-phase assays which take advantage of the affinity of the complexes for certain complement or rheumatoid factor proteins, and cellular assays which take advantage of the property of Raji cells to preferentially bind immune complexes; Theofilopoulos et al., chapter 28, and Toth et al., chapter 29, in Rose et al., eds. *Manual of Clinical Immunology*, 3rd Ed.

(American Society for Microbiology, Washington, D.C., 1986). Unfortunately, like many mammalian-cell based assays, the Raji-cell assay is difficult to perform, and requires elaborate controls and standards because of the inherent variability of Raji-cell binding to immune complexes.

In light of the foregoing, it would be advantageous for the medical community to have alternative assay methods for immune complexes utilizing well characterized, widely available, and conveniently cultured cell lines. It would also be advantageous if soluble Fc γ Rs could be produced in sufficient quantity to permit practical emergency therapy for refractory cases of ITP.

SUMMARY OF THE INVENTION

The present invention is directed to soluble and membrane-bound human Fc γ RIII polypeptides, their muteins, nucleic acids capable of encoding the same, and diagnostic and therapeutic uses of such polypeptides and muteins.

The invention is based on the discovery of a cDNA encoding a human Fc γ RIII. A cDNA clone, pcD(SR α) containing the Fc γ RIII-encoding insert (illustrated in Figure 1) is deposited in *E. coli* K12 strain MC1061 with the American Type Culture Collection (ATCC), Rockville, Maryland, USA, under accession number 67707.

Preferred embodiments of the invention include proteins having amino acid sequences selected from the group of 2-fold substituted and 1-fold deleted sequences defined by the formulas:

X(Arg) - X(Thr) - X(Glu) - X(Asp) - X(Leu) - X(Pro) - X(Lys) - X(Ala) - X(Val) - X(Val) - X(Phe) - X(Leu) - X(Glu) - X(Pro) - X(Gln) - X(Trp) - X(Tyr) - X(Arg) - X(Val) - X(Leu) - X(Glu) - X(Lys) - X(Asp) - X(Ser) - X(Val) - X(Thr) - X(Leu) - X(Lys) - X(Cys) - X(Gln) - X(Gly) - X(Ala) - X(Tyr) - X(Ser) - X(Pro) - X(Glu) - X(Asp) - X(Asn) - X(Ser) - X(Thr) - X(Gln) - X(Trp) - X(Phe) - X(His) - X(Asn) - X(Glu) - X(Asn) - X(Leu) - X(Ile) - X(Ser) - X(Ser) - X(Gln) - X(Ala) - X(Ser) - X(Ser) - X(Tyr) - X(Phe) - X(Ile) - X(Asp) - X(Ala) - X(Ala) - X(Thr) - X(Val) - X(Asp) - X(Asp) - X(Ser) - X(Gly) - X(Glu) - X(Tyr) - X(Arg) - X(Cys) - X(Gln) - X(Thr) - X(Asn) - X(Leu) - X(Ser) - X(Thr) - X(Leu) - X(Ser) - X(Asp) - X(Pro) - X(Val) - X(Gln) - X(Leu) - X(Glu) - X(Val) - X(His) - X(Val) - X(Gly) - X(Trp) - X(Leu) - X(Leu) - X(Leu) - X(Gln) - X(Ala) - X(Pro) - X(Arg) - X(Trp) - X(Val) - X(Phe) - X(Lys) - X(Glu) - X(Glu) - X(Asp) - X(Pro) - X(Ile) - X(His) - X(Leu) - X(Arg) - X(Cys) - X(His) - X(Ser) - X(Trp) - X(Lys) - X(Asn) - X(Thr) - X(Ala) - X(Leu) - X(His) - X(Lys) - X(Val) - X(Thr) - X(Tyr) - X(Leu) - X(Gln) - X(Asn) - X(Gly) - X(Lys) - X(Asp) - X(Arg) - X(Lys) - X(Tyr) - X(Phe) - X(His) - X(His) - X(Asn) - X(Ser) - X(Asp) - X(Phe) - X(His) - X(Ile) - X(Pro) - X(Lys) - X(Ala) - X(Thr) - X(Leu) - X(Lys) - X(Asp) - X(Ser) - X(Gly) - X(Ser) - X(Tyr) - X(Phe) - X(Cys) - X(Arg) - X(Gly) - X(Leu) - X(Val) - X(Gly) - X(Ser) - X(Lys) - X(Asn) - X(Val) - X(Ser) - X(Ser) - X(Glu) - X(Thr) - X(Val) - X(Asn) - X(Ile) - X(Thr)

- X(Ile) - X(Thr) - X(Gln) - X(Gly) - X(Leu) - X(Ala) -
X(Val) - X(Ser) - X(Thr) - X(Ile) - X(Ser) - X(Ser) - X(Phe)
- X(Ser) - X(Pro) - X(Pro) - X(Gly)

Formula 1

and

X(Arg) - X(Thr) - X(Glu) - X(Asp) - X(Leu) -
X(Pro) - X(Lys) - X(Ala) - X(Val) - X(Val) - X(Phe) -
X(Leu) - X(Glu) - X(Pro) - X(Gln) - X(Trp) - X(Tyr) -
X(Arg) - X(Val) - X(Leu) - X(Glu) - X(Lys) - X(Asp) -
X(Ser) - X(Val) - X(Thr) - X(Leu) - X(Lys) - X(Cys) -
X(Gln) - X(Gly) - X(Ala) - X(Tyr) - X(Ser) - X(Pro) -
X(Glu) - X(Asp) - X(Asn) - X(Ser) - X(Thr) - X(Gln) -
X(Trp) - X(Phe) - X(His) - X(Asn) - X(Glu) - X(Ser) -
X(Leu) - X(Ile) - X(Ser) - X(Ser) - X(Gln) - X(Ala) - X(Ser)
- X(Ser) - X(Tyr) - X(Phe) - X(Ile) - X(Asp) - X(Ala) -
X(Ala) - X(Thr) - X(Val) - X(Asp) - X(Asp) - X(Ser) -
X(Gly) - X(Glu) - X(Tyr) - X(Arg) - X(Cys) - X(Gln) -
X(Thr) - X(Asn) - X(Leu) - X(Ser) - X(Thr) - X(Leu) -
X(Ser) - X(Asp) - X(Pro) - X(Val) - X(Gln) - X(Leu) -
X(Glu) - X(Val) - X(His) - X(Ile) - X(Gly) - X(Trp) - X(Leu)
- X(Leu) - X(Leu) - X(Gln) - X(Ala) - X(Pro) - X(Arg) -
X(Trp) - X(Val) - X(Phe) - X(Lys) - X(Glu) - X(Glu) -
X(Asp) - X(Pro) - X(Ile) - X(His) - X(Leu) - X(Arg) -
X(Cys) - X(His) - X(Ser) - X(Trp) - X(Lys) - X(Asn) -
X(Thr) - X(Ala) - X(Leu) - X(His) - X(Lys) - X(Val) -
X(Thr) - X(Tyr) - X(Leu) - X(Gln) - X(Asn) - X(Gly) -
X(Lys) - X(Gly) - X(Arg) - X(Lys) - X(Tyr) - X(Phe) -
X(His) - X(His) - X(Asn) - X(Ser) - X(Asp) - X(Phe) -
X(Tyr) - X(Ile) - X(Pro) - X(Lys) - X(Ala) - X(Thr) - X(Leu)
- X(Lys) - X(Asp) - X(Ser) - X(Gly) - X(Ser) - X(Tyr) -
X(Phe) - X(Cys) - X(Arg) - X(Gly) - X(Leu) - X(Phe) -
X(Gly) - X(Ser) - X(Lys) - X(Asn) - X(Val) - X(Ser) -
X(Ser) - X(Glu) - X(Thr) - X(Val) - X(Asn) - X(Ile) - X(Thr)
- X(Ile) - X(Thr) - X(Gln) - X(Gly) - X(Leu) - X(Ala) -
X(Val) - X(Ser) - X(Thr) - X(Ile) - X(Ser) - X(Ser) - X(Phe)
- X(Phe) - X(Pro) - X(Pro) - X(Gly)

Formula II

wherein the term X(Xaa) represents the group of synon-
ymous L-amino acids to the amino acid Xaa. Synony-
mous amino acids within a group have sufficiently
similar physiochemical properties for substitution
between members of the group to preserve the biologi-
cal function of the molecule, Grantham, *Science*, Vol.
185, pgs. 862-864 (1974); and Dayhoff et al., *Atlas of*
Protein Sequence and Structure 1972, Vol. 5, pgs. 89-
99. It is clear that deletions of amino acids may also be
made in the above-identified sequence without altering
biological function, particularly if only a few amino acids
are deleted and amino acids that are critical to a func-
tional conformation are not removed or displaced, e.g.
some cysteine residues: Anfisen, "Principles That Gov-
ern The Folding of Protein Chains", *Science*, Vol. 181,
pgs. 223-230 (1973). Proteins and muteins produced by
deletions are within the purview of the present inven-
tion. Whenever an amino acid residue of a protein of
Formula I or II is referred to herein by number, such a
number is in reference to the N-terminus of the protein.

Preferred, and more preferred, groups of synony-
mous amino acids for each amino are listed in Table I.

The respective groups for each amino acid are indicated
by slashes (/). Amino acids to the left of the slash make
up the most preferred group. All the amino acids on the
same line make up the more preferred group.

As used herein, "N-fold substituted" in reference to
Formula I or II describes a group of amino acid
sequences differing from the native amino acid
sequences of Formula III or IV, respectively, by zero-N
substitutions, such that the replacing amino acid is
selected from the appropriate group of amino acids syn-
onymous to the amino acid at the location of the substi-
tution. That is, if the X(Ala) is at the location for
substitution, then the Ala at that location can be
replaced with Ser, Thr, or Gly.

Likewise, as used herein, the term "N-fold deleted"
in reference to Formula I or II describes a group of
amino acid sequences differing from the native amino
acid sequences of Formula III or IV, respectively, by
zero-N deletions of amino acids.

Throughout, standard abbreviations are used to
designate amino acids, nucleotides, restriction endonu-
cleases, and the like; e.g. Cohn, "Nomenclature and
Symbolism of α -Amino Acids," *Methods in Enzymology*,
Vol. 106, pgs. 3-17 (1984); Wood et al. *Biochemistry: A*
Problems Approach, 2nd ed. (Benjamin, Menlo Park,
1981); and Roberts, "Directory of Restriction Endonu-
cleases", *Methods in Enzymology*, Vol. 68, pgs. 27-40
(1979).

Table I

Preferred and More Preferred Groups of Synonymous Amino Acids	
Amino Acid	Synonymous Groups
Ala	Ala Ser / Thr Gly
Arg	Arg / Lys
Asn	Asn Asp / Ser
Asp	Asp Glu / Asn
Cys	Cys
Gln	Gln / Glu
Glu	Glu Asp / Gln
Gly	Gly Ala / Ser
His	His / Asn
Ile	Ile Val / Leu
Leu	Leu Val / Ile
Lys	Lys Arg / Asn
Met	Met / Leu
Phe	Phe
Pro	Pro / Ala
Ser	Ser Ala Thr / Gly Asn
Thr	Thr Ser Ala / Lys
Trp	Trp
Tyr	Tyr
Val	Val Ile / Ala Leu

Brief Description of The Drawings

Figure 1, parts A and B (on two sheets to be read side-by-side) illustrates the nucleotide sequence of the cDNA insert of pcD(SR α)-GP5;

Figure 2 illustrates the relative location of the stop codon inserted to produce a soluble human Fc γ RIII, secreted as an Fc γ RIII mutant;

Figure 3 illustrates the result of gel electrophoretic analysis of the soluble Fc γ RIII produced in Example III below; and

Figure 4 parts A and B (on two sheets to be read side-by-side) illustrates the nucleotide sequence of the cDNA insert of pcD(SR α)-NL10.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes nucleic acids encoding polypeptides capable of binding to the Fc portion of human IgG. These polypeptides are derived from

human Fc γ RIII. The invention includes soluble and membrane-bound polypeptides for human Fc γ RIII. These and other modified versions of the polypeptides are readily produced using standard protein engineering techniques.

Once nucleic acid sequence and/or amino acid sequence information is available for a native protein, a variety of techniques become available for producing virtually any mutation in the native sequence. Shortle, in *Science*, Vol. 229, pgs. 1193-1201 (1985), reviews techniques for mutating nucleic acids which are applicable to the present invention. Preferably, mutants of the protein of the present invention are produced by site-specific oligonucleotide-directed mutagenesis, e.g. Zoller and Smith, *Methods in Enzymology*, Vol. 100, pgs. 468-500 (1983), and Mark et al., U.S. Patent 4,518,584 entitled "Human Recombinant Interleukin-2 Muteins", which are incorporated by reference; or by so-called "cassette" mutagenesis described by Wells et al. in *Gene*, Vol. 34, pgs. 315-323 (1985), by Estell et al. in *Science*, Vol. 233, pgs. 659-663 (1986), and also essentially by Mullenbach et al. in *J. Biol. Chem.*, Vol. 261, pgs. 719-722 (1986), and by Feretti et al. in *Proc. Natl. Acad. Sci.*, Vol. 83, pgs. 597-603 (1986).

Polypeptides with amino acid modifications (i.e. muteins) may be desirable in a variety of circumstances. For example, undesirable side effects might be reduced by certain muteins, particularly if the side effect is associated with a different part of the polypeptide from that of the desired activity. In some expression systems, the native polypeptide may be susceptible to degradation by proteases. In such cases, selected substitutions and/or deletions of amino acids which change the susceptible sequences can significantly enhance yields, e.g. British patent application 2173-804-A where Arg at position 275 of human tissue plasminogen activator is replaced by Gly or Glu. Muteins may also increase yields in purification procedures and/or increase shelf lives of proteins by eliminating amino acids susceptible to oxidation, acylation, alkylation, or other chemical modifications. For example, methionine readily undergoes oxidation to form a sulfoxide, which in many proteins is associated with loss of biological activity: e.g. Brot and Weissbach, *Arch. Biochem. Biophys.*, Vol. 223, pg. 271 (1983). Methionines can often be replaced by more inert amino acids with little or no loss of biological activity, e.g. Australian patent application AU-A-52451/86. In bacterial expression systems, yields can sometimes be increased by eliminating or replacing conformationally inessential cysteine residues, e.g. Mark et al., U.S. Patent 4,518,584.

Preferably, soluble forms of the Fc γ RIII of the invention are produced by introducing a stop codon prior to (i.e. in the 5'- or "upstream" direction of) the coding region for the transmembrane and intracellular portions of the Fc γ RIII cDNA. This is conveniently done by site-specific mutagenesis. Transmembrane regions are readily identified by the presence of an amino acid segment containing from about 20-25 residues having high

average hydrophobicity, e.g. Wickner, *Science*, Vol. 210, pgs. 861-868 (1980), and Greene et al., *Ann. Rev. Immunol.*, Vol. 4, pgs. 69-95 (1986).

Plasmid pcD(SR α)-GP5 is similar to the pcD shuttle vector described by Okayama and Berg, *Mol. Cell. Biol.*, Vol. 2, pgs. 161-170 (1983), and Vol. 3, pgs. 280-289 (1983), except that the SV40 promoter has been modified to improve expression by the downstream insertion of a portion of the long terminal repeat (LTR) from a HTLV(I) retrovirus, described by Takebe et al., *Mol. Cell. Biol.*, Vol. 8, pgs. 466-472 (1988). The plasmid is conveniently propagated in *E. coli* K12 strain MC1061, or like host.

The immunoglobulin G binding property of the Fc γ RIII of the invention is measured by standard techniques, e.g. (1) in the case of membrane-bound Fc γ RIII: the ability of cells transfected with the Fc γ RIII cDNA to form rosettes in the presence of IgG-coated red blood cells (RBCs) or to preferentially bind human IgG aggregated by heat treatment; or (2) in the case of soluble Fc γ RIII: the ability to preferentially remove Fc γ RIII from solution by an immunoadsorbent column comprising human IgG, or to inhibit rosette formation between IgG-coated RBCs and cells known to have Fc γ RIII. The former measurements can be made with fluorescently labeled human IgG molecules, e.g. Haugland, *Handbook of Fluorescent Probes* (Molecular Probes, Inc., Junction City, OR, 1985). The latter measurements can be made by constructing an IgG column from isolated human IgG and a commercially available activated sepharose column, e.g. from Bio-Rad Laboratories (Richmond, CA).

Rosette assays are standard in the art, e.g. Winchester et al., chapter 31, in Rose et al., eds., *Manual of Clinical Laboratory Immunology*, 3rd Ed. (American Society for Microbiology, Washington, D.C., 1986).

Once the cDNA of the invention has been cloned, a wide range of expression systems (i.e. combinations of host and expression vector) can be used to produce the proteins of the invention. Possible types of host cells include, but are not limited to, bacterial, yeast, insect, mammalian, and the like. Selecting an expression system, and optimizing protein production thereby, involves the consideration and balancing of many factors, including (1) the nature of the protein to be expressed, e.g. the protein may be poisonous to some host organisms, it may be susceptible to degradation by host proteases, or it may be expressed in inactive conformations or in insoluble form in some hosts, (2) the nature of the messenger RNA (mRNA) corresponding to the protein of interest, e.g. the mRNA may have sequences particularly susceptible to host endonucleases, which drastically reduce the functional lifetime of the mRNA, or the mRNA may form secondary structures that mask the start codon or ribosome binding site, thereby inhibiting initiation of translation in some hosts, (3) the selection, availability, and arrangement of host-compatible expression-control sequences in the 3'- and 5'-regions flanking the coding region -- these include promoters, 5'- and 3'-

protector sequences, ribosome binding sites, transcription terminators, enhancers, polyadenylate addition sites, cap sites, intron-splice sites, and the like, (4) whether the protein has a secretion-signal sequence which can be processed by the host, or whether an expression-control sequence encoding a signal sequence endogenous to the host must be spliced onto the region encoding the mature protein, (5) the available modes and efficiencies of transfection or transformation of the host, and whether transient or stable expression is desired, (6) the scale and cost of the host culture system desired for expressing the protein, (7) whether, and what type of, posttranslational modifications are desired, e.g. the extent and kind of glycosylation desired may affect the choice of host, (8) the ease with which the expressed protein can be separated from proteins and other materials of the host cells and/or culture medium e.g. in some cases it may be desirable to express a fusion protein with a specialized signal sequence to aid in later purification steps, e.g. Sassenfeld et al., *Biotechnology*, January 1984, (9) the stability and copy number of a particular vector in a selected host, e.g. Hofschneider et al., eds. *Gene Cloning in Organisms Other than E. coli* (Springer Verlag, Berlin, 1982), and (10) like factors known to those skilled in the art.

Many reviews are available which provide guidance for making choices and/or modifications of specific expression systems in light of the recited factors, e.g., de Boer and Shepard, "Strategies for Optimizing Foreign Gene Expression in *Escherichia coli*" pgs. 205-247, in Kroon, ed. *Genes: Structure and Expression* (John Wiley & Sons, New York, 1983), review several *E. coli* expression systems; Kucherlapati et al., *Critical Reviews in Biochemistry*, Vol. 16, Issue 4, pgs. 349-379 (1984), and Banerji et al., *Genetic Engineering*, Vol. 5, pgs. 19-31 (1983) review methods for transfecting and transforming mammalian cells; Reznikoff and Gold, eds., *Maximizing Gene Expression* (Butterworths, Boston, 1986) review selected topics in gene expression in *E. coli*, yeast, and mammalian cells; and Thilly, *Mammalian Cell Technology* (Butterworths, Boston, 1986) reviews mammalian expression systems.

Likewise, many reviews are available which describe techniques and conditions for linking and/or manipulating specific cDNAs and expression control sequences to create and/or modify expression vectors suitable for use with the present invention: e.g. Maniatis et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, N.Y., 1982); Glover, *DNA Cloning: A Practical Approach*, Vol. I and II (IRL Press, Oxford, 1985), and Perbal, *A Practical Guide to Molecular Cloning* (John Wiley & Sons, N.Y., 1984).

Suitable expression systems for the invention include those disclosed by Itakura and Riggs, U.S. patent 4,704,362 (bacterial expression), by Clark et al., U.S. patent 4,675,285 and by Hamer U.S. patent 4,599,308 (mammalian expression), and by Kurjan et al., U.S. patent 4,546,082 (yeast expression). Accord-

ingly, the above patents are incorporated by reference.

Whenever SV40-based vectors are used, e.g. pcD vectors, a preferred host is the COS7 cell line, described by Gluzman, Cell, Vol. 23, pgs. 175-182 (1981) and available from the ATCC under accession number CRL1651.

Soluble Fc γ RIII of the invention are administered as a pharmaceutical composition for treating ITP. Such compositions contain an effective amount of the Fc γ RIII in a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivering the compositions of the invention to a patient. Generally, compositions useful for parenteral administration of such drugs are well known, e.g. Remington's Pharmaceutical Science, 15th Ed. (Mack Publishing Company, Easton, PA 1980). Alternatively, compositions of the invention may be introduced into a patient's body by an implantable drug delivery system, e.g. Urquhart et al., Ann. Rev. Pharmacol. Toxicol., Vol. 24, pgs. 199-236 (1984).

When administered parenterally, the soluble Fc γ RIII will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutical carrier. Such carriers are inherently non-toxic and non-therapeutic. Examples of such carriers are normal saline, Ringer's solution, dextrose solution, and Hank's solution. Nonaqueous carriers such as fixed oils and ethyl oleate may also be used. A preferred carrier is 5% dextrose/saline. The carrier may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The soluble Fc γ RIII is preferably formulated in purified form substantially free of aggregates and other proteins at a concentration in the range of about 5 to 30 mg/ml, and preferably at a concentration in the range of about 10 to 20 mg/ml.

Selecting an administration regimen to deliver to a patient an amount of soluble Fc γ RIII which is effective in ameliorating the thrombopenia associated with ITP depends on several factors, including the serum turnover rate of the soluble Fc γ RIII, the serum level of competing endogenous Fc γ RIII associated with the immune disorder, the possible immunogenicity of the soluble Fc γ RIII, and the like. Preferably, an administration regimen maximizes the amount of soluble Fc γ RIII delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of soluble Fc γ RIII delivered depends in part on the particular soluble Fc γ RIII employed and the severity of the disease being treated. Guidance in selecting appropriate doses is found in the literature on therapeutic uses of antibodies and antibody fragments, which are polypeptides of roughly the same size as the soluble Fc γ RIII, e.g. Bach et al., chapter 22, in Ferrone et al., eds., Handbook of Monoclonal Antibodies (Noges Publications, Park Ridge, NJ, 1985); and Russell, pgs. 303-357, and Smith et al., pgs. 365-389, in Haber et al., eds., Antibodies in Human Diagnosis and Therapy (Raven Press, New York, 1977). Preferably, the dose is in the range of about 1-20 mg/kg

per day, more preferably about 1-10 mg/kg per day.

EXAMPLES

The following examples serve to illustrate the present invention. Selection of vectors and hosts as well as the concentration of reagents, temperatures, and the values of other variables are only to exemplify application of the present invention and are not to be considered limitations thereof.

Example I. Construction of stable mammalian cell transformants which express human Fc γ RIII

Preferably, mammalian cell lines capable of stable expression of the Fc γ RIII are produced by cotransfecting a host mammalian cell with a vector carrying a selectable marker and a vector carrying a host-compatible promoter and the Fc γ RIII cDNA insert. For pcD(SR α)-GP5, suitable hosts include Chinese hamster ovary cells, COS monkey cells, and mouse L cells, such as a thymidine kinase deficient mutant (tk⁻) L cell available from the American Type Culture Collection under accession number CCL 1.3. The selectable marker allows one to select host cells which have a high probability of containing the Fc γ RIII gene fully integrated into the host genome. Typically, the ratio of pcD(SR α)-GP5 to the marker-containing vector in the transfection solution is about 10:1. Thus, if the marker gene is integrated into the host genome, it is very likely that pcD(SR α)-GP5 will also be integrated by virtue of its higher concentration. The selectable marker also provides a means of preventing the cultures of desired transformants from being overgrown by revertant cells.

tk⁻ mouse L cells are cotransfected with pcD(SR α)-GP5 and pSV2tk, a pSV2 plasmid carrying a thymidine kinase gene under control of the SV40 early promoter. The pSV2 plasmid is described by Mulligan et al., Science, Vol. 209, pgs. 1422-1427 (1980), and by Subramani et al., Mol. Cell. Biol., Vol. 1, pgs. 854-864 (1981), and is available from the American Type Culture Collection under accession number 37146. Both plasmids are amplified in E. coli, e.g. strain HB101 available from the ATCC under accession number 33694, and purified by cesium chloride equilibrium centrifugation. A suspension of about 1×10^5 of tk⁻ L cells in 10 ml of Dulbecco's Modified Eagle Medium (DME) with 10% fetal bovine serum is placed in a Falcon 3003 dish and cultured at 37°C for 20 hours in a 5% carbon dioxide gas incubator, after which the medium is replaced by 10 ml of fresh DME with 10% fetal bovine serum. The culture is incubated for an additional 4 hours. After incubation, 0.5 ml of solution A (50 mM Hepes, 280 mM NaCl, 1.5 mM sodium phosphate buffer, pH 7.22) and 0.5 ml of solution B (2M CaCl₂, 10 μ g pcD(SR α)-GP5, 1 μ g pSV2tk) are added to the culture medium, and the culture is incubated at 37°C for 24 hours in a 5% CO₂ atmosphere, after which the cells are placed in a selective medium with HAT (e.g. Sigma Chemical Co., St. Louis,

MO). After two weeks the surviving colonies are subcloned by limiting dilution, and clones are assayed for expression of FcγRIII.

Example II. Use of stable L cell transformant expressing membrane-bound FcγRIII to measure serum levels of immune complex

A stably transformed mammalian cell expressing the FcγRIII of the invention can replace the Raji cell line in assays for immune complexes, e.g. Theofilopoulos et al., chapter 28, *Manual of Clinical Laboratory Immunology*, 3rd Ed. (American Society for Microbiology, Washington, D.C. 1986).

Antiserum to human IgG is prepared in rabbits, and the IgG fraction is isolated by ammonium sulfate precipitation followed by fractionation on an anion-exchange chromatography column (DEAE-cellulose 52; Whatman Chemical Separation Ltd., Maidstone, England). Antiserum from commercial sources may also be used. The IgG fraction of the antiserum is brought to 5 mg/ml, and 1 ml is labeled with ¹²⁵I. After iodination and dialysis, the antiserum is diluted to 1 mg/ml with phosphate-buffered saline (PBS) to give a specific activity of about 3 x 10⁵ cpm/μg. Cells transformed with the FcγRIII cDNA are harvested after 72 hours of culture, and portions of 2 x 10⁶ cells in 200 μl of medium are placed in 1.5-ml plastic Eppendorf conical tubes (Brinkmann Instruments, Inc., Westbury, N.Y.; catalog no. 22-36-411-1). One ml. of Spinner medium (Eagle minimal medium without Ca²⁺ and Mg²⁺) is added to each tube, and the cells are centrifuged at 800 x g for 8 min. Supernatant fluids are aspirated, and the cell pellets are resuspended in 50 μl of Spinner medium. Serum to be tested is diluted fourfold in 0.15 M NaCl (physiological saline), and 25 μl is added to the transformed cells. After a 45-min incubation period at 37°C with gentle shaking by hand every 5 to 10 min, the cells are washed three times with Spinner medium. After the final wash, the cells, gently shaken every 5 to 10 minutes, are allowed to react for 30 min at 4°C with ¹²⁵I-labeled rabbit anti-human IgG diluted with Spinner medium containing 10 g of human serum albumin (HSA) per liter. After incubation, the cells are washed three times, supernatant fluids are completely aspirated, and radioactivity of the cell pellet is determined in a gamma counter. All assays are done in duplicate. The amount of uptake, expressed as absolute counts, percentage of the input, or micrograms of antibody, is referred to a standard curve of radioactive antibody uptake by cells incubated with normal human serum (complement source) containing various amounts of aggregated human IgG (AHG). The quantity of immune complex in serum is equated to an amount of AHG after correction for the dilution factor and is expressed as micrograms of AHG equivalent per milliliter of serum. The soluble AHG is formed from a solution of 6.5 mg of Cohn fraction II or isolated human IgG per ml of physiological saline, heated at 63°C for 30 min, and centrifuged (1,500 x g, 15 min) to remove insoluble

large aggregates. The supernatant is then diluted with buffer to yield a final concentration of approximately 1.6 mg/ml. Portions (0.5 ml) of this preparation are stored at -70°C and can be used for as long as 1 month.

The standard curve of radioactive antibody uptake is constructed as follows. Fifty-microliter portions of AHG (80 μg of protein) are serially diluted (11 twofold dilutions) in saline. Subsequently, 50 μl of a twofold dilution of normal human serum (source of complement), freshly obtained or stored at -70°C, is added to each dilution of AHG, mixed carefully, and incubated at 37°C for 30 min. Thereafter, 25 μl of each mixture is added to 2 x 10⁶ cells in duplicate (fourfold final dilutions of serum containing from 20 μg to about 20 ng of AHG); the mixture is incubated, washed, and reacted with radiolabeled antibody. Radioactivity is then counted as with the test sera. A base line of radioactive antibody uptake (background) by cells incubated with 25 μl of a fourfold dilution of normal human serum, used as a source of complement in the reference curve, is also established.

Standard preparations of IgG aggregates and of tetanus toxoid-human anti-tetanus toxoid immune complexes have recently been developed under the auspices of the International Union of Immunologists and are available through the Swiss Red Cross Blood Transfusion Service, e.g. Nydegger et al., *Clin. Exp. Immunol.*, Vol. 58, pgs. 502-509 (1984).

Example III. Construction of a soluble human FcγRIII

Soluble FcγRIII was constructed using site-specific oligonucleotide-directed mutagenesis of the FcγRIII cDNA insert of pcD(SRα)-GP5. The entire cDNA insert of pcD(SRα)-GP5 was subcloned as a 2.4 kilobase Bam HI fragment into the Bluescript KS plasmid (Stratagene, San Diego, CA) and single-stranded DNA was then prepared.

A synthetic oligonucleotide (minimum size about 18 nucleotides) encoding a TAA stop codon and a Bam HI site (with mutations shown in boldface in Figure 2) was used as a primer for complementary strand synthesis using DNA polymerase I large fragment (Amersham, Arlington Heights, IL). Figure 2 indicates the position of the stop codon and Bam HI site relative to the major domains encoded by the FcγRIII cDNA: S represents the region encoding the signal peptide, EC represents the region encoding the extracellular domain of FcγRIII, H represents the region encoding the hydrophobic, or transmembrane, domain of FcγRIII, and C represents the region encoding the cytoplasmic domain. Introducing the stop codon as indicated by Figure 2 produces a mutant FcγRIII lacking both the cytoplasmic and transmembrane domains; it is soluble as shown by the designation "sFcγRIII" beside the modified DNA sequence. After the identity of the mutant was confirmed by DNA sequence analysis, it was subcloned back into the Bam HI site of the pcD(SRα) vector, amplified in *E. coli*, and transfected into COS 7 cells by electroporation. One day prior to transfection, approximately (1.5-2.0) x 10⁶

COS 7 monkey cells were seeded onto individual 100 mm plates in Dulbecco's modified Eagle's medium (DME) containing 6% fetal calf serum and 2 mM glutamine. To perform the transfection, cells were harvested by trypsinization and counted, washed twice in serum-free DME, and suspended to $(1-7) \times 10^6$ cells per ml in serum-free DME. DNA was added to 25 μ g/ml and the mixture was allowed to stand at room temperature for 10 minutes, after which 0.8 ml was pulsed in a 0.4 cm sterile cuvette with a Bio Rad (Richmond, CA) Gene Pulser at 250 volts and 960 microfarads. After pulsing, cells were allowed to stand for 10 minutes and then were plated at $(1.5-2.0) \times 10^6$ per 100 mm plate in DME plus 6% fetal calf serum. Supernatants were harvested and assayed for soluble Fc γ RIII after 72 hours.

The soluble Fc γ RIII in the COS7 culture supernatants was analyzed further by gel electrophoresis, and immunoprecipitated. The results of this are illustrated in Figure 3. Lanes 1, 3 and 6 each contain proteins from culture supernatants of COS7 cells transfected with the pcD carrying the soluble Fc γ RIII cDNA. Lane 1 proteins were immunoprecipitated with a non-specific mouse IgG₂ antibody; Lane 3 proteins were immunoprecipitated with human IgG, presumably by the binding of the Fc portion of the antibody with the soluble Fc γ RIII; and Lane 6 proteins were immunoprecipitated with the monoclonal antibody 3G8 which is specific for the extracellular domain of Fc γ RIII. Size markers adjacent to the Control lane (lane 1) are in kilodaltons. The soluble Fc γ RIII corresponds to the broad band at about 40 kilodaltons. Lanes 4 and 7 contain proteins from culture supernatants of COS7 cells transfected with the pcD carrying the soluble Fc γ RIII cDNA and cultured in the presence of tunicamycin. Tunicamycin inhibits the post-translational attachment of N-linked carbohydrates to proteins. It was applied in an attempt to determine the nature of the apparent heterogeneity of the 40 kilodalton band. As shown in Figure 2, the tunicamycin causes the appearance of two bands of lower molecular weight, which is consistent with the deglycosylation of the soluble Fc γ RIII. Tunicamycin was added to the cultures as disclosed by Martens et al., PNAS 84: 809-813 (1987). Lanes 2 and 5 contain proteins from culture supernatants of COS7 cells which had undergone the same manipulations as the COS cells of lanes 3 and 4 and lanes 6 and 7, respectively, with the exception that no plasmid DNA was included in the transfection protocol.

Example IV Isolation of a Variant human Fc γ RIII from Natural Killer Cells.

A cDNA library was constructed from mRNA extracted from a human natural killer (NK) cell line using the pcD(SR α) expression vector. The library was screened with a probe constructed from the cDNA insert of pcD(SR α)-GP5, which was radiolabeled with ³¹P by random-primed DNA labeling (Boehringer Mannheim, Indianapolis, IN). A clone pcD(SR α)-NL10 was obtained that exhibited human IgG binding activity. The

sequence of the cDNA insert of pcD(SR α)-NL10 is illustrated in Figure 4. It can be seen that the amino acid sequence of this Fc γ RIII and that encoded by pcD(SR α)-GP5 are very similar, differing in the extracellular domain by only six amino acids.

The descriptions of the foregoing embodiments of the invention have been presented for purpose of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in light of the above teaching. The embodiments are chosen and described in order to best explain the principles of the invention and its practical application to thereby enable others skilled in the art to best utilize the invention in various embodiments and with such modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

Applicants have deposited pcD(SR α)-GP5 with the American Type Culture Collection Rockville, MD, USA (ATCC), under accession number 67707. This deposit is made under the Budapest Treaty for the Deposit of Microorganisms; and also under conditions as provided under ATCC's agreement for Culture Deposit for Patent Purposes, which assures that the deposit will be made available to the US Commissioner of Patents and Trademarks pursuant to 35 U.S.C. 122 and 37 C.F.R. 1.14, and will be made available to the public upon issue of a U.S. patent, which requires that the deposit be maintained. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Claims

1. Soluble or membrane-bound human Fc γ RIII.
2. Fc γ RIII according to claim 1 having the amino acid sequence of Formula I or II.
3. A variant of Fc γ RIII according to claim 2, wherein the Fc γ RIII has the sequence of Formula I or II with 0 or 1 amino acid residue deleted and 0, 1 or 2 amino acid residues substituted according to the following table, and wherein there is at least one deletion or at least 1 substitution:

amino acid in Formula I or II	substituted by
Ala	Ser, Thr or Gly
Arg	Lys
Asn	Asp or Ser
Asp	Glu or Asn
Gln	Glu
Glu	Asp or Gln
Gly	Ala or Ser
His	Asn
Ile	Val or Leu
Leu	Val or Ile
Lys	Arg or Asn
Met	Leu
Pro	Ala
Ser	Ala, Thr, Gly or Asn
Thr	Ser, Ala or Lys
Val	Ile, Ala or Leu.

5. A protein consisting of the extracellular domain of a human Fc γ RIII.

5 6. A pharmaceutical composition comprising an effective amount of Fc γ RIII according to any of Claims 1-4 or a protein according to Claim 5, and a pharmaceutically acceptable carrier.

10 7. A pharmaceutical composition according to Claim 6 for treatment of immune thrombocytopenic purpura.

8. A pharmaceutical composition according to Claim 6 or Claim 7 comprising 5-30 mg/ml Fc γ RIII.

15 9. A pharmaceutical composition according to Claim 6 or Claim 7 comprising 10-20 mg/ml Fc γ RIII.

10. Use of Fc γ RIII in manufacture of a medicament for treatment of thrombocytopenia.

20 11. Use of Fc γ RIII in manufacture of a medicament for treatment of immune thrombocytopenic purpura.

25 12. Use according to Claim 10 or Claim 11 of Fc γ RIII according to any of Claims 1-4 or of a protein according to Claim 5.

30

4. A variant of Fc γ RIII according to claim 2, wherein the Fc γ RIII has the sequence of Formula I or II with 1 or 2 amino acid residues substituted according to the following table:

35

amino acid in Formula I or II	substituted by
Ala	Ser
Asn	Asp
Asp	Glu
Glu	Asp
Gly	Ala
Ile	Val
Leu	Val
Lys	Arg
Ser	Ala or Thr
Thr	Ser or Ala
Val	Ile.

40

45

50

55

10 30 50
 CACTCCAGTGTGGCATCATGTGGCAGCTGCTCCTCCCAACTGCTCTGCTACTT
 MetTrpGlnLeuLeuLeuProThrAlaLeuLeuLeu
 110 130 150
 GTTCCTGGAGCCTCAATGGTACAGGGTGCTCGAGAAGGACAGTGTGACTCTG
 lPheLeuGluProGlnTrpTyrArgValLeuGluLysAspSerValThrLeu
 210 230 250
 CACAATGAGAACCTCATCTCAAGCCAGGCCTCGAGCTACTTCATTGACGCTGCC
 HisAsnGluAsnLeuIleSerSerGlnAlaSerSerTyrPheIleAspAlaAla
 310 330 350
 CCCTCAGTGACCCGGTGCAGCTAGAAGTCCATGTCCGGCTGGCTGTTGCTCCAG
 hrLeuSerAspProValGlnLeuGluValHisValGlyTrpLeuLeuLeuGln
 410 430 450
 TCACAGCTGGAAGAACACTGCTCTGCATAAGGTCACATATTTACAGAATGGC
 sHisSerTrpLysAsnThrAlaLeuHisLysValThrTyrLeuGlnAsnGly
 510 530 550
 GCCACACTCAAAGATAGCGGCTCCTACTTCTGCAGGGGGCTTGTGGGAGTAAA
 AlaThrLeuLysAspSerGlySerTyrPheCysArgGlyLeuValGlySerLys
 610 630 650
 CAGTGTCAACCATCTCATCTCTCTCCACCTGGGTACCAAGTCTCTTTCTGC
 laValSerThrIleSerSerPheSerProProGlyTyrGlnValSerPheCys
 710 730 750
 TGTGAAGACAAACATTTGAAGCTCAACAAGAGACTGGAAGGACCATAAACTTA
 rValLysThrAsnIleEnd

AATAAGAGCAGTGGCAGCAGCATCTCTGAACATTTCTCTGGATTTGCAACCCC
 GCCAGATCCTTTATCCAACCTCTCGATTTTTCCTTGGTCTCCAGTGGAAGGGAA
 CTAGAAATTGAAGTTTCAGAGCTACACAAACACTTTTTCTGTCCCAACCATTC
 CATACAAAAATTGCTCGTATTATAAATTACCCAGTTTAGAGGGGAAAAAAGAA
 AGGACCCTACAGAGTGTGGGAACTGCTGGGGATCTAGAGAATTCAGTGGGACC
 GGGAGGTGTTCCCATCTGAGCCCAGAGATAAGGGTGTCTTCCTAGAACATTAG
 CTTCCAGGGGACTCTATCAGAACTGGACCATTTCOAAGTATATAACGATGAGC
 TTGGGGTGGGGTGGGGTGGAAAAGAAAGTACAGAACAAACCCCTGTGTCACTG
 AATGAGAAAGCCTGAGAAGAAAGAACCAACCACACAGGAAGGAAAGC
 TTAATGCAGGGACTGTAAAACCACTTTTCTGCTTCAATGTCTAGTTCTCTGTA
 TAAATGTACTACTGAGCTTCACTGAGTTACGCTGTGAACTTTCAAATCCTTC
 ATGAAAAAAGCTTTAGCTGTCTCCTGTTTTGTAAGCTTTCAGTGCAACATTT

FIGURE 1 PART A

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          70   +1                               90
CTAGTTTCAGCTGGCATGCCGACTGAAGATCTCCCAAAGGCTGTGGT
LeuValSerAlaGlyMetArgThrGluAspLeuProLysAlaValVa
          170                               190
AAGTGCCAGGGAGCCTACTCCCCTGAGGACAATTCCACACAGTGGTTT
LysCysGlnGlyAlaTyrSerProGluAspAsnSerThrGlnTrpPhe
          270                               290
ACAGTCGACGACAGTGGAGAGTACAGGTGCCAGACAAACCTCTCCA
ThrValAspAspSerGlyGluTyrArgCysGlnThrAsnLeuSerT
          370                               390
CCCCCTCGGTGGGTGTTCAAGGAGGAAGACCCTATTCACCTGAGGTG
AlaProArgTrpValPheLysGluGluAspProIleHisLeuArgCy
          470                               490
AAAGACAGGAAGTATTTTCATCATAATTCTGACTTCCACATTCCAAAA
LysAspArgLysTyrPheHisHisAsnSerAspPheHisIleProLys
          570                               590
AATGTGTCTTCAGAGACTGTGAACATCACCATCACTCAAGGTTTGG
AsnValSerSerGluThrValAsnIleThrIleThrGlnGlyLeuA
          670                               690
TTGGTGATGGTACTCCTTTTTGCAGTGGACACAGGACTATATTTCTC
LeuValMetValLeuLeuPheAlaValAspThrGlyLeuTyrPheSe
          770                               790
AATGGAGAAAGGACCCTCAAGACAAATGACCCCCATCCCATGGGAGT

ATCATCCTCAGGCCTCTCTACAAGCAGCAGGAAACATAGAACTCAGA
AAGCCCATGATCTTCAAGCAGGGAAGCCCCAGTGAGTAGCTGCATTC 1000
CCTCACAGTAAACAACAATACAGGCTAGGGATGGTAATCCTTTAAA
AATAATTATTCCTAAACAAATGGATAAGTAGAATTAATGATTGAGGC 1200
AATGAAAGCATGGCTGAGAAATAGCAGGGTAGTCCAGGATAGTCTAA
CCGTAGTGGAATTAACAGGAAATCATGAGGGTGACGTAGAATTGAGT 1400
CCTCTAATGCTAGGAGTAGCAAATGGTCCTAGGAAGGGGACTGAGGA
TCCCAAGTTAAGCTAAGTGAACAGAACTATCTCAGCATCAGAATGAG 1600
GCAGGAGGTGAAAATGCTTTCTTGGCCAGGGTAGTAAGAATTAGAGG
TAGCTTTGTTTCATTGCATTTATTAAACAAATGTTGTATAACCAATAC 1800
TTCAGTCAGTTCCAATGAGGTGGGGATGGAGAAGACAATTGTTGCTT
CTTGGTTCCAATAAAGCATTTTACA (A)n

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FIGURE 1 PART B

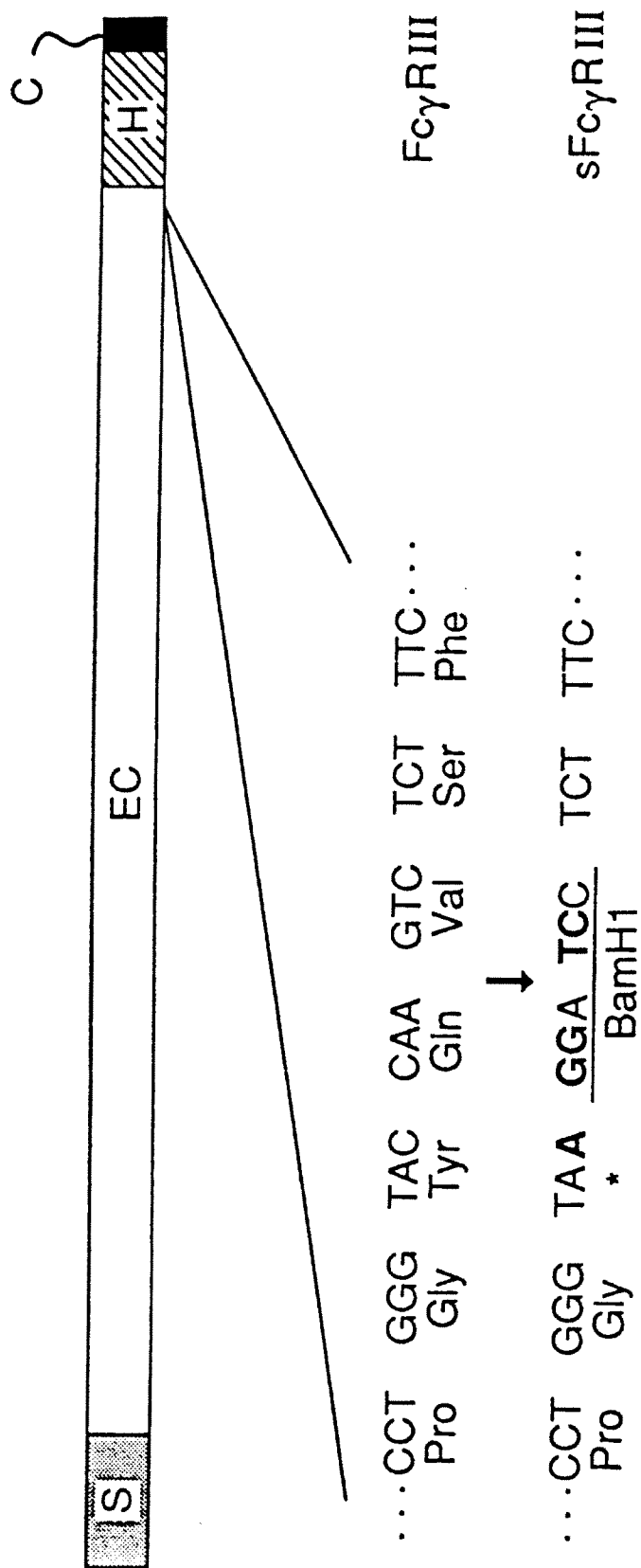


FIGURE 2

NL 10

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      10      30      50
GGGGGGGGGGGGGGTAAATCCGCAGGACCTGGGTAACACGAGGAAGGGCTCCG

      110      130      150
TGCTCCTCCCAACTGCTCTGCTACTTCTAGTTTCAGCTGGCATGCGGACTGAA
euLeuLeuProThrAlaLeuLeuLeuLeuValSerAlaGlyMetArgThrGlu

      210      230      250
GCTCGAGAAGGACAGTGTGACTCTGAAGTGCCAGGGAGCCTACTCCCTGAG
lLeuGluLysAspSerValThrLeuLysCysGlnGlyAlaTyrSerProGlu

      310      330      350
GCCTCGAGCTACTTCATTGACGCTGCCACAGTCCGACGACAGTGGAGAGTACAGG
AlaSerSerTyrPheIleAspAlaAlaThrValAspAspSerGlyGluTyrArg

      410      430      450
TCCATATCGGCTGGCTGTTGCTCCAGGCCCTCGGTGGGTGTTCAAGGAGGAA
alHisIleGlyTrpLeuLeuLeuGlnAlaProArgTrpValPheLysGluGlu

      510      530      550
TAAGGTCACATATTTACAGAATGGCAAAGGCAGGAAGTATTTTCATCATAAT
sLysValThrTyrLeuGlnAsnGlyLysGlyArgLysTyrPheHisHisAsn

      610      630      650
TTCTGCAGGGGGCTTTTTGGGAGTAAAAATGTGTCTTCAGAGACTGTGAACATC
PheCysArgGlyLeuPheGlySerLysAsnValSerSerGluThrValAsnIle

      710      730      750
CACCTGGGTACCAAGTCTCTTTCTGCTTGGTGATGGTACTCCTTTTTGCAGTG
roProGlyTyrGlnValSerPheCysLeuValMetValLeuLeuPheAlaVal

      810      830      850
AAGAGACTGGAAGGACCATAAAATTTAAATGGAGAAAGGACCCTCAAGACAAATGA
rArgAspTrpLysAspHisLysPheLysTrpArgLysAspProGlnAspLysEnd

GAACATTTCTCTGGATTTGCAACCCCATCATCCTCAGGCCTCTCTACAAGCAGCA
TTTCCTTGGTCTCCAGTGGAAGGGAAAAAGCCCATGATCTTCAAGCAGGGAAGCCC
AAACACTTTTTCTGTCCCAACCGTTCCCTCACAGCAAAGCAACAATACAGGCTAG
TACCCAGTTTAGAGGGGAAAAAAAAACAATTATTCCTAAATAAATGGATAAGTAG
GGGATCTAGGGAATTCAGTGGGACCAATGAAAGCATGGCTGAGAAATAGCAGGTA
AAGGGTGTCTTCTAGAACATTAGCCGTAGTGGAATTAACAGGAAATCATGAGGG
ATCTCCAAGTATATAACGATGAGTCCTCTTAATGCTAGGAGTAGAAAAATGGTCCT
AGTACAGAACAAACCCCTGTGTCACTGTCCCAAGTTGCTAAGTGAACAGAACTATC
GCACACAGGAAGGGGGGGCGCAGGAGGTGAAAATGCTTTCTTGGCCAGGGTAGTAA
CAATATCTAATTCCTGTGTAGCTTTGTTTCATTGCATTTATTAAACAAATGTTGTA
GAAACTTTCAAATCCTTCATCATGTCAAGTCCAATGAGGTGGGGATGGAGAAGAC
GCTTTAAGCGCAACATTTCTTGGTTCCAATAAAGCATTTTACAAGATCTTGCATG
ATGATAAAAAAAAAAAAAAAAAAAAA

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FIGURE 3 PART A

NL 10

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      70      90
GATATCTTTGGTGA CTTGTCCACTCCAGTGTGGCATCATGTGGCAGC
                               MetTrpGlnL
      170      190
GATCTCCCAAAGGCTGTGGTGTTCCTGGAGCCTCAATGGTACAGGGT
AspLeuProLysAlaValValPheLeuGluProGlnTrpTyrArgVa
      270      290
GACAATTCCACACAGTGGTTTCACAATGAGAGCCTCATCTCAAGCCAG
AspAsnSerThrGlnTrpPheHisAsnGluSerLeuIleSerSerGln
      370      390
TGCCAGACAAAACCTCTCCACCCTCAGTGACCCGGTGCAGCTAGAAG
CysGlnThrAsnLeuSerThrLeuSerAspProValGlnLeuGluV
      470      490
GACCCTATTACCTGAGGTGTCACAGCTGGAAGAACA C TGTCTGCA
AspProIleHisLeuArgCysHisSerTrpLysAsnThrAlaLeuHi
      570      590
TCTGA CTTCTACATTCCAAAAGCCACACTCAAAGACAGCGGCTCCTAC
SerAspPheTyrIleProLysAlaThrLeuLysAspSerGlySerTyr
      670      690
ACCATCACTCAAGGTTTGGCAGTGTCAACCATCTCATCATTCTTTTC
ThrIleThrGlnGlyLeuAlaValSerThrIleSerSerPhePheP
      770      790
GACACAGGACTATATTTCTCTGTGAAGACAAACATT CGAAGCTCAAC
AspThrGlyLeuTyrPheSerValLysThrAsnIleArgSerSerTh
      870      890
CCCCCATCCCATGGGGGTAATAAGAGCAGTAGCAGCAGCATCTCT

GGAAACATAGAACTCAGAGCCAGATCCCTTATCCAAC TCTCGACT      1000
CAGTGAGTAGCTGCATTCCCTAGAAAATTGAAGTTTCAGAGCTACAC
GGATGGTAATCCCTTTAAACATACAAAAATTGCTCGTCTTATAAAT      1200
AATTAATGGTTGAGGCcAGGACCATACAGAGTGTGGGAACTGCTG
GTCCAGGATAGTCTAAGGGAGGTGTTCCCATCTGAGCCCAGAGAT      1400
TGACGTAGAATTGAGTCTTCCAGGGGACTCTATCAGAACTGGACC
AGGAAGGGGACTGAGGATTGCGGTGGGGGGTGGGGTGGAAAAGAA      1800
TCAGCATCAGAA TGAGAAAGCCTGAGAGAAAGAACCAACCACAA
GAATTAGAGGTTAATGCAGGGACTGTAAAACCACTTTTCTGCTT      1800
TAACCAATACTAAATGTACTACTGAGCTTCGCTGAGTTAAGTTAT
AATTGTTGCTTATGAAAGAAAGCTTTACGTGTCTCTGTTTTGTAA      2000
CTACTCTTAGATAGAAGATGGGAAAACCATGGTAATAAAATATGA

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FIGURE 3 PART B



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 97 10 6398
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	WO 88 06733 A (KHAYAT DAVID) 7 September 1988 * claims 1-5,20 *	1,2,5-12	C12N15/12 C07K14/735 A61K38/17
P,X	NATURE, JUN 9 1988, 333 (6173) P568-70, ENGLAND, XP002032511 SIMMONS D ET AL: "The Fc gamma receptor of natural killer cells is a phospholipid-linked membrane protein [published erratum appears in Nature 1989 Aug 24;340(6235):662]" * the whole document *	1,2,5-12	
A	J IMMUNOL METHODS, JUN 26 1987, 100 (1-2) P235-41, NETHERLANDS, XP002032512 KHAYAT D ET AL: "Soluble circulating Fc gamma receptors in human serum. A new ELISA assay for specific and quantitative detection." * the whole document *	1,2,5,12	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C12N C07K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		6 June 1997	Nauche, S
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO FORM 1503 03.82 (P0407)



European Patent Office

EP 97106398 - C -

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely: 1,2,5,10-12
Claims searched incompletely: 6-9
Claims not searched: 3,4

Reason for the limitation of the search:

The amount of possible combinations of all variants disclosed in claims 3 and 4 is speculative : for either Formula, there are more than $3 \cdot 10^{14}$ possible combinations in claim 3 and more than $2 \cdot 10^{16}$ possible combinations in claim 4. These claims do not meet the requirements for article 84 EPC.